

The diagram illustrates the three-step PCR process for generating a pool of mutant *argA* genes. It shows the progression from the wild-type (*wt*) *ArgA* gene to a pool of mutant genes through three PCR steps, each introducing a mutation (indicated by a notch in the DNA strand).

- Initial state:** The *ArgA* gene, *wt* (wild-type).
- First PCR step:** Amplification using primers P1 and P2. A mutation is introduced (indicated by a notch in the DNA strand).
- Second PCR step:** Amplification using primers P2 and P3. A second mutation is introduced (indicated by a notch in the DNA strand).
- Third PCR step:** Amplification using primers P2 and P3. A third mutation is introduced (indicated by a notch in the DNA strand). Restriction sites for *Nco*I and *Hind*III are indicated.
- Final state:** Pool of mutant *argA* genes.